African Journal of Biotechnology Vol. 9(31), pp. 4885-4889, 2 August, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Antibacterial activity of metal complexes of antifolate drug pyrimethamine

Omoruyi G. Idemudia and Peter A. Ajibade*

Department of Chemistry, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa.

Accepted 16 March, 2010

Ag(I), Co(II) and Cu(II) complexes of pyrimethamine [5-(4-chlorophenyl)-6-ethyl pyrimidinediamine] have been synthesized and characterized by elemental analysis, conductivity measurement, FTIR and UV-Vis spectroscopy. Pyrimethamine act as a monodentate ligand through the pyrimidinic N(1) atom and coordinate the metal ions to give rise to a 4-coordinate tetrahedral and 6-coordinate octahedral for the Co(II) and Cu(II) complexes and linear coordination geometry for the Ag(I) complexes. The *in vitro* antimicrobial activities of the complexes against some bacteria were determined using the macro dilution method. The Ag(I) complexes showed enhanced activity and their minimum inhibitory concentration and minimum bacteria concentration were determined.

Key words: Pyrimethamine, metal complexes, antimicrobial activity, antibiotic, drug resistance.

INTRODUCTION

Compounds containing pyrimidine rings play a significant role in biological systems (Saha and Kar, 1977). Pyrimethamine is an antifolate drug widely used in malarial chemotherapy. It inhibits the bacterial enzymes dihydrofolate reductase (Macreadie et al., 2000; Simo et al., 2000). Pyrimethamine is generally administered in conjunction with a sulphonamide resulting in a synergistic effect due to the sequential inhibition of enzymatic steps in the folate synthesis provided by the combination (Sirawaraporn and Yuthavong, 1986; Chulay et al., 1984). It is an effective drug against protozoan parasites, such as *Toxoplasma gondi* and *Plasmodium falciparum*, is poorly water soluble, weakly basic (pka 7.34) and exhibit marked variation in oral bioavailability (Onyeji et al., 2009). At present, disease-causing microbes that have become resistant to antibiotic drug therapy are on the increase and resistance against antimicrobial agents is becoming a public health problem worldwide (Levy, 1998; Cohen, 1992; Neu, 1992). In the search for novel therapy against resistant organism, the modification of existing drug by combination to a metal centre has gained attention in recent years (Navarro et al., 1997; Delhaes et al., 2001).

The efficacies of some therapeutic agents are known to increase upon co-ordination, thus metal-based drug is seen as promising alternatives for possible replacement for some of the current drugs. A number of antibiotics such as bleomycin, streptonigrin and bactracin have been reported to function properly upon coordination with metal ions (Li-June, 2003). Metal complexes as pharmaceuticals have received considerable attention in the development of anticancer agents using platinum, ruthenium and other metals, with greater efficacy and reduced toxic side effects (Timerbaev et al., 2006). Vanadium compounds, either alone or in combination with other agents, have the potential to serve as anti-diabetic agents (Srivastava 1995). Studies on Cu(II) with pyrimethamine have been reported and the complex showed some activity against resistant strain of *Plasmodium falciparum* and *T*rypanosoma *brucei rhodosience* (Ajibade and Kolawole, 2008). The recognition of the potential employment of metal complexes and chelates in therapeutic application provides useful outlet for basic research in transition metal chemistry (Obaleye et al., 1997) and in line with this application we present the synthesis, characterization and antimicrobial studies of Ag(I), Co(II) and Cu(II) complexes of pyrimethamine drug.

*Corresponding author. E-mail: pajibade@ufh.ac.za. Tel: +27 40 602 2055. Fax: +27 40 602 2094.

MATERIALS AND METHODS

Materials

All metal salts, reagents and chemicals are of analytical grade and they were used without further purification. Melting point determination was carried out using GallenKamp melting point apparatus, conductivity measurement was carried out using CON 6/TDS 6 Hand-held conductivity/TDS Meter. FTIR spectra were obtained on a Perkin-Elmer Lambda 25 Model System 2000 FT-IR spectropho-tometer in the range 4000 - 370 cm^{-1}. UV-Vis spectra was obtained on an Elmer Lambda 25 UV-VIS spectrophotometer. *Staphyloco-ccus aureus* (ATCC 6538), *Streptococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 10702), *Bacillus pumilus* (ATCC 14884), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 19582), *Enterobacter cloacae* (ATCC 13047), and *Proteus vulgaris* (CSIR 0030) typed cultures as obtained from the American Typed Culture Collection (ATCC) were used.

Synthesis of the metal complexes

Pyrimethamine (4 mmol, 0.995 g) was dissolved in 50 mL of methanol and heated slightly for the drug to dissolve completely. This was followed by drop-wise addition of corresponding metal salts in methanol in 2:1 mole ratios for Cu(II) and Co(II) complexes and 1:1 for silver complexes. In some cases, 1 mole of diphenylphosphinoethane (DPPE) was added. The resultant solution in each case was refluxed for 6 h, evaporated under reduced pressure and the precipitate filtered off and washed with methanol and diethylether and dried in vacuum.

Antimicrobial study

The metal complexes were dissolved in minimum amount of dimethylformamide and diluted with water. Mueller-Hinton agar medium suitable for testing the sensitivity of clinically important pathogens towards antibiotics, and nutrient broth were prepared with the standard preparatory techniques. Typed culture organisms comprising gram positive and gram negative bacteria isolates were sub-cultured (Broth innoculation of organisms) and placed in an incubator with temperature of 37 - 39°C for 18 - 24 h. Sensitivity testing to ascertain the activities of the metal complexes was determined using agar well diffusion method as described (Irobi et al., 1994; Russell and Furr, 1977) with little modifications.

Minimum inhibitory concentration (MIC)

Duplicates of two fold serial dilution of complexes were prepared from a solution of metal complexes in sterilized distilled water. 2 ml of different concentration of the prepared solutions was added to 18 ml of pre-sterilized molten nutrient agar at a temperature of 50°C to give final concentrations of 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625 and 0.0078125 mg/mL. The medium was poured to sterile Petri-dishes to allow it to set. The dry surface of the nutrient agar medium was then streaked with sub-cultured bacterial isolates. Plates were incubated after streaking, in an incubator at 37°C for 24 h, and examined for the presence or absence of growth (Akinpelu and Kolawole, 2004). MIC was taken as the lowest concentration that will prevent the bacteria growth.

Minimum bacteria concentrations (MBC)

MBC of metal complexes were determined using (Olorundare et al., 1992). Samples of organisms were taken from plates which were used for the MIC test that were with no visible growth and sub cultured on to freshly prepared nutrient agar medium by streaking. These plates were incubated at 37°C for 24 h. The MBC was taken as the lowest concentration of the complexes that did not allow any bacterial growth on the surface of the agar plates.

RESULTS

The analytical data for the complexes are presented in Table 1, percentage yield, melting point / decomposition temperature and conductivity measurement of the complexes as well as their colour are presented in Table 2. The physical properties of the complexes showed that their colour varied from grey-white for the silver complexes to pink and green for copper and cobalt complexes. The complexes are stable solid with melting point ranging from 160 - 238°C. Conductivity measurements of the complexes as presented in Table 2 showed that the complexes are non-electrolytes in solution. Selected infrared bands for the metal complexes and the pyrimethamine drug are presented in Table 3. A comparison of the IR spectrum of the ligand with the metal complexes showed that the N-H band in the pyrimethamine experience little or no change in the complexes while the C=N bond shifted in the complexes as compared to the ligand. The electronic spectra of the complexes also confirm the coordination of the metal ions to pyrimethamine and are in accord with the geometries proposed for the complexes. The *in vitro* antimicrobial screening of the complexes showed that the Co(II) and Cu(II) complexes does not possess any antimicrobial activity while the Ag(I) complexes have some antimicrobial properties. The zones of inhibition of the Ag(I) complexes are presented in Table 4. The minimum inhibitory concentration (MIC) and the minimum bacteria concentration (MBC) of these complexes were determined. The results are presented in Tables 5 and 6.

DISCUSSION

Metal complexes of pyrimethamine drug were prepared by the reaction of the appropriate metal salts with pyrimethamine drug in methanol. The complexes were isolated as air stable solids. Elemental analysis revealed the composition of the metal complexes. All the metal complexes melted/ decomposed below 250°C and their solubility studies showed that they are generally insoluble in both polar and non-polar solvent but soluble in polar coordinating solvents such as dimethylsulfoxide and dimethylformamide. The conductivity measurements in DMF at 10^{-3} $moldm^{-3}$ showed that the complexes are non-electrolyte in solution. The analytical data, colour, melting point/ decomposition temperature, molecular formula and conductivity data of the complexes are presented in Table 1. Ag(I) complexes formed a two coordinate specie

Table 1. Analytical data of complexes

Compound	Molecular formula	Formula weight	Analytical data found (Calculated)		
			C	H	N
$[Ag(pyrm)_2Cl]\cdot 3H_2O$	$C_{24}H_{32}N_8Cl_3O_3Ag$	694.79	41.64 (41.49)	3.99 (4.64)	16.74 (16.13)
$[Ag(pyrm)CH_3COO]$	$C_{14}H_{16}N_4ClO_2Ag$	415.63	40.66 (40.46)	3.25 (3.88)	13.36 (13.48)
$[Cu(pyrm)_2Cl_2]\cdot H_2O$	$C_{24}H_{28}N_8Cl_4OCu$	649.89	43.80 (44.35)	4.45 (4.34)	17.60 (17.24)
$[Cu(pyrm)_2(CH_3COO)_2]$	$C_{28}H_{32}N_8Cl_2O_4Cu$	679.06	50.22 (49.52)	4.87 (4.75)	18.27 (16.50)
$[Cu(pyrm)_2(CH_3COO)_2DPPE]\cdot H_2O$	$C_{54}H_{58}N_8Cl_2O_5P_2Cu$	1095.49	58.81 (59.20)	5.18 (5.34)	10.42 (10.23)
$[Co(pyrm)_2(CH_3COO)_2DPPE]\cdot 3H_2O$	$C_{54}H_{62}N_8Cl_2O_7P_2Co$	1126.91	57.16 (57.55)	5.24 (5.55)	10.23 (9.94)
$[Co(pyrm)_2Cl_2DPPE]\cdot 2H_2O$	$C_{50}H_{54}N_8Cl_4O_2P_2Co$	1061.71	56.91 (56.56)	4.97 (5.13)	10.74 (10.55)

Table 2. Some physical properties of the complexes.

Compound	Colour	M.P/D.T (°C)	Cond. (µS)	(%) Yield
$[Ag(pyrm)_2Cl]\cdot CH_3OH$	Gray	238	3.70	97
$[Ag(pyrm)CH_3COO]$	White	199	60.80	74
$[Cu(pyrm)_2Cl_2]\cdot H_2O$	Purple	218	46.70	96
$[Cu(pyrm)_2(CH_3COO)_2]$	Green	208	2.77	95
$[Cu(pyrm)_2(CH_3COO)_2DPPE]\cdot H_2O$	Green	160	13.99	89
$[Co(pyrm)_2(CH_3COO)_2DPPE]\cdot 3H_2O$	Pink	236	4.19	98
$[Co(pyrm)_2Cl_2DPPE]\cdot 2H_2O$	Dark Green	206	43.60	97

Table 3. Relevant infrared frequencies (cm^{-1}) for the ligand and the complexes.

Compound	*v*(C=N)	*v*(N-H)s	*v*(N-H)as	*v*(CO_2)
Pyrimethamine	1576	3468	3311	
$[Ag(pyrm)_2Cl]\cdot CH_3OH$	1559	3468	3312	
$[Ag(pyrm)CH_3COO]$	1578	3468	3339	1597, 1433
$[Cu(pyrm)_2Cl_2]\cdot H_2O$	1560	3441	3315	
$[Cu(pyrm)_2(CH_3COO)_2]$	1560	3468	3316	1478
$[Cu(pyrm)_2(CH_3COO)_2DPPE]\cdot H_2O$	1560	3468	3313	1479
$[Co(pyrm)_2(CH_3COO)_2DPPE]\cdot 3H_2O$	1562	3440	3394	1479
$[Co(pyrm)_2Cl_2DPPE]\cdot 2H_2O$	1577	3440	3312	

by coordinating to one molecule of pyrimethamine, while the Co(II) and Cu(II) complexes formed a tetrahedral and octahedral coordination depending on the reactants involved in the synthesis process, with the metal coordinating with two molecules of pyrimethamine each (Figure 2).

The IR spectra of the ligands and complexes were compared and assigned on the basis of careful comparison Table. The N-H stretching frequencies of the pyrimethamine molecule undergoes a slight shift in all the complexes. These could be attributed to the overlap of u(N-H) and u(O-H) of methanol or inter and intra molecular hydrogen bonding (Ajibade and Kolawole, 2008). The pyrimidinic N(1) atom which which appear as

Table 4. Sensitive patters of zones of inhibition exhibited by complexes on some pathogens.

Microorganism	$[Ag_2(pyrm)_2Cl]\cdot 3H_2O$ mg/ml	$[Ag(pyrm)CH_3COO]$ mg/ml
Staphylococcus aureus	20.5	22.0
Streptococcus faecalis	18.5	21.5
Bacillus cereus	10.5	15.5
Bacillus pumilus	9.5	16.5
Escherichia coli	9.0	14.0
Pseudomonas aeruginosa	8.0	18.5
Enterobacter cloacae	15.5	19.5
Proteus vulgaris	7.5	12.5

Table 5. The minimum inhibitory concentrations (MIC) exhibited by the complexes against the bacteria isolates.

Microorganism	$[Ag_2(pyrm)_2Cl]\cdot 3H_2O$ mg/ml	$[Ag(pyrm)CH_3COO]$ mg/ml
Staphylococcus aureus	0.03125	0.0625
Streptococcus faecalis	0.03125	0.0625
Bacillus cereus	0.03125	0.0625
Bacillus pumilus	0.03125	0.0625
Escherichia coli	0.03125	0.0625
Pseudomonas aeruginosa	0.03125	0.0625
Enterobacter cloacae	0.03125	0.0625
Proteus vulgaris	0.0625	0.0625

a prominent single band at 1576 cm^{-1} in the parent drug shift to different position in the complexes. This may be attributed to the fact that coordination of the parent drug to the metal ions preferentially takes place through the N(1) atom (Ajibade et al, 2006). The presence of the acetate ion in some complexes is confirmed by bands at about 1479 cm^{-1} (Simo et al; 2000; Ajibade et al 2007).

The silver complexes occur as a two coordinate linear species. Their electronic spectra do not have a band in the visible region as a result of the absence of a d - d transition which is indicative of a d^{10} Ag(I) configuration. Ag(I) complexes possess a transition band at about 250 nm which is a characteristics of a metal charge 4d - 4s transition at relatively high energies (Kunkely and Vogler, 2006). The electronic spectra of $[Cu(pyrm)_2$ (CH_3COO) DPPE$]\cdot H_2O$ showed a broad band in the region 700 nm expected for a d - d transition of an octahedral Cu(II) complex. The broadness of the band could be attributed to the overlapping of several bands as a result of strong Jahn-Teller distortion expected in a d^9 ion (Ajibade et al., 2006). In the electronic spectra of the Co(II) complexes, $[Co(pyrm)_2(Cl)_2$ DPPE$]\cdot 2H_2O$ show two bands of weak to medium intensity at 600 nm and 690 nm respectively which may tentatively be assigned to $^4T_{1g}$ (F) $\rightarrow$ $^4T_{1g}$ and $^4T_{1g}$ (F) $\rightarrow$ $^4T_1$ (P) transitions of pseudo-octahedral cobalt(II). (Ajibade et al., 2006). $[Co(pyrm)_2$ (CH_3COO) DPPE$]\cdot 3H_2O$ shows a single weak transition at about 450 nm which can be attributed to the d - d transition of a d^7 Co(II).

Antimicrobial test

Selectivity test of metal complexes on bacteria isolates revealed that the silver complexes possess antimicrobial activities compared to the pyrimethamine drug which did not show any activity. The Ag(I) complexes exhibited activities against all eight bacterial isolates comprising of both gram-positive and gram-negative organisms. These showed that the Ag(I) complexes possess a broad spectrum activity. *P. vulgaris* shows the least zone of inhibition with 7.5 mm and 12.5 mm in $[Ag(pyrm)_2$ $Cl]\cdot 3H_2O$ and $[Ag(pyrm)CH_3COO]$ respectively. While *Staphylococcus aureus* has the highest zone of inhibition with 20.5 mm and 22 mm for $[Ag(pyrm)_2Cl]\cdot 3H_2O$ and $[Ag(pyrm)CH_3COO]$ respectively as shown in Table 4. The MIC of the silver complexes as presented in Table 5 shows that $[Ag_2(pyrm)_2Cl]\cdot 3H_2O$ has an MIC value of 0.03125 mg/mL against all the organisms except for *P. vulgaris* with a value of 0.0625 mg/ml. And for the complex $[Ag(pyrm)CH_3COO]$, the MIC value against all the organism is 0.0625 mg/ml. This results indicated that $[Ag(pyrm)_2Cl]\cdot 3H_2O$ has stronger activity. The MBC (minimum bacteria concentration) value which is the lowest concentration of the metal complex that will prevent the bacteria growth was performed. $[Ag(pyrm)_2Cl]\cdot 3H_2O$

Table 6. The minimum bacteria concentrations (MBC) exhibited by the complexes against the bacteria isolates

Microorganism	$[Ag(pyrm)_2Cl]\cdot 3H_2O$ mg/ml	$[Ag(pyrm)CH_3COO]$ mg/ml
Staphylococcus aureus	0.03125	0.0625
Streptococcus faecalis	0.03125	0.0625
Bacillus cereus	0.03125	0.0625
Bacillus pumilus	0.03125	0.0625
Escherichia coli	0.03125	0.0625
Pseudomonas aeruginosa	0.03125	0.0625
Enterobacter cloacae	0.0625	0.0625
Proteus vulgaris	0.0625	0.0625

has MBC value of 0.03125 mg/mL against all bacteria isolate except *Enterobacter cloacae* and *P. vulgaris* with 0.0625 mg/mL MBC value. $[Ag(pyrm)CH_3COO]$ has MBC value of 0.0625 mg/mL against all the bacteria isolates as shown in Table 5. This result along with MIC results further reveals that $[Ag(pyrm)_2Cl]\cdot 3H_2O$ has a stronger antimicrobial activity.

Conclusion

Air stable Ag(I), Co(II) and Cu(II) complexes of pyrimethamine have been synthesized and characterized. Based on their electronic spectral results, a linear geometry has been suggested for the Ag(I) complexes, tetrahedral geometry and an octahedral geometry for Co(II) and Cu(II) complexes. The antimicrobial screening of the complexes showed that the Ag(I) complexes have enhanced activity, with $\{[Ag_2(pyrm)_2].0.7CH_3OH\}$ showing a stronger antimicrobial potential at an MIC value of 0.03125 mg/ml.

ACKNOWLEDGEMENT

The authors would like to thank Govan Mbeki Research and Development Centre, University of Fort Hare for her financial support.

REFERENCES

Ajibade PA, Kolawole GA. (2008). Synthesis Characterization and antiprotozoal studies of some metal complexes of antimalarial drugs, Transition Met Chem (33): 493-497

Ajibade PA, Kolawole GA, Brien PO (2007). Metal Complexes of 4-Amino-N-(2-pyrimidinyl)benzene Sulfonamide: Synthesis, Characterization and Antiprotozoal Studies. Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chem, (37): 653-659.

Ajibade PA, Kolawole GA, Brien PO, Helliwell M, Raftery J (2006). Cobalt(II) complexes of the antibiotic sulfadiazine, the X-ray single crystal structure of $[Co(C_{10}H_9N_4O_2S)_2(CH_3OH)_2]$, Inorgan. Chim. Acta (359) 3111-3116

Akinpelu DA, Kolawole DO (2004). Phytochemistry and antimicrobial activity of leaf extract of *Piliostigma thonningii* (Schum). Sci. Focus. 7: 64-70.

Chulay JD, Watkins WM, Sixsmith DG (1984). Synergistic antimalarial activity of pyrimethamine and sulfadoxine against *Plasmodium falciparum in vitro*. Am. J. Trop. Med. Hyg. 33: 325-330.

Cohen ML (1992). Epidemiology of drug resistance: implications for a post-antimicrobial era. Sci. 257: 1050-1055.

Delhaes L, Abessolo H, Biot C, Berry L, Delcourt P, Maciejewski L, Brocard J, Camus D, Dive D (2001). *In vitro* and *in vivo* antimalarial activity of ferrochloroquine, a ferrocenyl analogue of chloroquine against chloroquine-resistant malaria parasites. Parasitol. Res. 87: 239.

Irobi ON, Moo Young M, Anderson WA. (1994). Antimicrobial activity of Annato (*Bixa orellana*) extract. Int. J. Pharmacog. 34: 87-90

Kunkely H, Vogler A (2006) Photo properties of silver sulfadiazine, Inorganic Chemistry Communications (10): 226-228.

Levy SB (1998) The Challenge of Antibiotic Resistance. Scientific American.

Li-june M (2003) Structure and function of metallo-antibiotics. Med. Res. Rev. 6(23): 697-762.

Navarro M, Perez H, Sanchez-Delgado RA (1997). Toward a novel metal based chemotherapy against tropical diseases 3. Synthesis and antimalarial activity *in vitro* and *in vivo* of the new gold-chloroquine complex [Au(PPh3)(CQ)]PF6. J. Med. Chem. 40, pp. 1937-1939.

Neu HC (1992). *The* crisis in antibiotic resistance. Sci. 257: 1064-1073.

Olorundare EE, Emudianughe TS, Khasar GS, Koteyi SA, Irobi N (1992). Antibacterial properties of leave extract of *Cassia alata*. Biol. Res. Com. 4: 13-117.

Onyeji CO, Omoruyi SI, Oladimeji FA, Soyinka JO (2009). Physicochemical characterization and dissolution properties of binary systems of pyrimethamine and 2-hydroxypropyl-β-cyclodextrin. Afr. J. Biotechnol. 8(8): 1651-1659.

Russell AD, Furr JR (1977). The antimicrobial activity of a new chloroxylenol preparation containing ethylenediamine tetraacetic acid, J. Appl. Bacteriol. 43: 253.

Saha N, Kar Sk (1977) Metal complexes of pyrimidine-derived ligands—I : Nickel(II) complexes of 2-hydrazino-4,6-dimethyl pyrimidine, J. Inorg. Nucl. Chem., 39(1): 195-200.

Simo B, Porello L, Ortiz R, Castineiras A, Latorre J, Canton E (2000). Interactions of metal ions with a 2,4-diaminopyrimidine derivative (trimethoprim) Antimicrobial studies. J. Inorganic. Biochem. 81. 275-283.

Sirawaraporn W, Yuthavong (1986). Potentiating Effect of Pyrimethamine and Sulfadoxine against Dihydrofolate Reductase from Pyrimethamine-Sensitive and Pyrimethamine-Resistant *Plasmodium chabaudi*. Antimicrob. Agent Chemother. 29(5): 899-905.

Srivastava AK (1995). Potential use of vanadium compounds in the treatment of diabetes mellitus. Exp. Opin. Invest Drugs 4: 525-536.

Timerbaev AR, Hartinger CG, Keppler BK (2006) Metallodrug research and analysis using capillary electrophoresis. TrAC Trends Anal. Chem. 25(9): 868-875.